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Stability of Minute Virus of Mice to Chemical and Physical Agents

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Minute virus of mice (MVM), a single-stranded deoxyribonucleic acid Parvovirus, was subjected to various inactivation procedures, including chemical disinfectants, heat, and ultraviolet radiation. MVM was found to be less stable than has been reported for other Parvoviruses. This virus was readily inactivated by a variety of chemical disinfectants, including alcohols, formaldehyde, glutaraldehyde, and chloroform. MVM was more sensitive to ultraviolet radiation than was Kilham's rat virus. MVM was more sensitive to heating at temperatures of 35 to 100 C than has been reported for other Parvoviruses. More than 95% of MVM infectivity was inactivated by heating (45, 60, or 100 C) for 60 min, acid (pH 2.0) treatment, or ultraviolet radiation treatment, although a small percentage (less than 2%) of the virus preparation was found to be resistant to these treatments. In addition, more than 99% of the infectivity of MVM was lost after storage at 4C for 10 weeks, although the virus was stable on storage in liquid nitrogen.

Minute virus of mice (MVM) is a Parvovirus indigenous to murine species (3, 6). Under natural conditions, MVM is transmitted from animal to animal by direct contact or by exposure to contaminated fomites. In the laboratory, MVM is prevalent in murine breeding colonies and has been reported to be a common contaminant of cell cultures and tumor suspensions prepared from murine species (2, 6, 8). Although MVM is a considerable problem in experiments with murine species, few data are available concerning methods of inactivating this virus with chemical agents, heat, or ultraviolet (UV) light energy (3, 9). The current study was designed to investigate the stability of MVM to these treatments. The results indicate that MVM is readily inactivated by common chemical disinfectants and is relatively sensitive to thermal or UV light or acid inactivation, although a "resistant population" of MVM persists after these last three treatments.

MATERIALS AND METHODS

Virus. MVM was obtained through the courtesy of John C. Parker, Microbiological Associates, Bethesda, Md. For this study, a single virus pool was prepared from infected secondary rat embryo cell (REC) cultures (13, 14). The virus pool was dispensed in small portions and stored in liquid nitrogen. The 50% tissue culture infectivity dose ($TCID_{50}$) was $10^{6.7}$/ml in secondary REC, and the hemagglutinin titer was 1:512 with guinea pig erythrocytes (3, 9). The virus pool was identified as MVM by neutralization of cell culture infectivity and hemagglutination-inhibition tests, using specific MVM antisera (Microbiological Associates).

Cell cultures. Pregnant rats (Flow Laboratories, Dublin, Va.) were serologically tested in our laboratory and determined to be free of evidence of MVM infection. A single pool of REC was prepared from 15- to 18-day decapitated and eviscerated rat embryos and stored as a 10% suspension in small portions in liquid nitrogen. Primary cultures were grown in Eagle minimal essential medium with Earle balanced salt solution, containing 10% fetal calf serum, 200 U of penicillin per ml, 100 μg of streptomycin per ml, and 10 μg of fungizone per ml. For viral assays, secondary REC cultures were prepared in 24-well trays (Linbro, New Haven, Conn.) by seeding cultures with 7×10^5 cells/ml and incubating in 2% CO_2 at 36 C.

Viral assays. MVM was assayed in secondary REC according to the methods of Parker et al. (9). Briefly, growth medium was removed from 75 to 85% confluent monlolayers, and cells were inoculated in quadruplicate with 0.1 ml of tenfold dilutions of virus in growth medium. Uninfected controls were inoculated with growth medium and, in appropriate experiments, with 0.1 ml of the chemical agent. After adsorption for 2 h at 36 C, the cells were washed twice with Hanks balanced salt solution, and maintenance medium consisting of Wistar Eagle minimal essential medium (15), 2% fetal calf serum, and antibiotics was added. Cells were observed microscopically for cytopathic effect daily for 12 days. Cultures showing 2+ or greater cytopathic effect were used

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to calculate the viral titer ($TCID_{50}$) by the method of Reed and Muench (12).

Stability studies. For chemical inactivation studies, a 1:10 dilution of MVM was made in the test chemical. The chemicals included 95% or 70% ethanol, 99% isopropanol, 1.0 or 0.5% formaldehyde, 5.25% sodium hypochlorite, 2% glutaraldehyde (Cidex, Arbrook, Arlington, Tex.), and undiluted chloroform. Concentrated chemicals were diluted in deionized water to achieve the desired concentration. After completion of the treatments, individual samples were assayed for residual MVM.

For acid inactivation, samples of 1 ml of MVM were placed in sterile ¼-inch (ca. 0.64 cm) dialysis tubing and dialyzed against 0.15 N KCl-HCl buffer at pH 2.0 at 4 C for 11 days. At various days, samples were taken, the pH was adjusted to pH 7.2 by dialysis against 0.02 M Na_2HPO_3 in Hanks balanced salt solution, and the samples were assayed for residual MVM.

For temperature inactivation, 1-ml samples of MVM in 12- by 75-mm polypropylene tubes were subjected to various temperatures, including: −196 C (liquid nitrogen), 4 C (refrigerator), and 35, 45, 60, and 100 C (water baths). Samples were taken at varying time periods from initial exposure and assayed for residual virus.

For UV inactivation, 1-ml samples of MVM in 100- by 15-mm open plastic petri dishes were placed on a rocker platform and irradiated for varying periods with a General Electric UV germicidal lamp (principal emission, 254 nm) at an average dose rate of 20 ergs per mm^2 per s. Total UV doses of between 100 and 500 ergs/mm^2 were delivered to the experimental groups, and 500 ergs/mm^2 were delivered to the control consisting of growth medium alone.

For all stability studies, the degree of inactivation was in reference to the original virus pool, which titered $10^{6.7}$ $TCID_{50}$/ml.

RESULTS

Inactivation of MVM by chemical disinfectants. MVM infectivity was markedly reduced by all of the disinfectants tested (Table 1). After exposure to formaldehyde, glutaraldehyde, or alcohol solutions, the infectivity of the virus was reduced by greater than $10^{5.7}$ $TCID_{50}$/ml. Treatment of the virus suspension with chloroform or sodium hypochlorite reduced the infectivity titer by at least $10^{4.7}$ $TCID_{50}$/ml; inactivation greater than this level could not be determined, since these two disinfectants at high concentrations were toxic for secondary REC.

In contrast to the sensitivity to these chemical disinfectants, MVM was partially resistant to inactivation by acid conditions (Fig. 1). Exposure of the virus to pH 2.0 required several days to reduce the viral titer to $10^{1.3}$ $TCID_{50}$/ml, although 99% of the infectivity was lost in 1 day. After 11 days, residual infectivity could not be detected.

Thermal stability of MVM. MVM was found to be relatively heat sensitive (Fig. 2). When virus preparations (which orginally titered $10^{6.7}$ $TCID_{50}$/ml) were heated at 100 C for 15 min, infectivity of the virus could not be detected. When virus was heated at 60 C for 60 min, the virus titer was reduced to $10^{2.6}$ $TCID_{50}$/ml. With treatment at 45 C for 120 min, the

TABLE 1. *Inactivation of MVM by chemical disinfectants*[a]

Chemical	Concn	Reduction of titer from control[b] (TCID/ml)
Ethanol	95%	$\geq 10^{5.7}$
Ethanol	70%	$\geq 10^{5.7}$
Isopropanol	99%	$\geq 10^{5.7}$
Formaldehyde	1.0%	$\geq 10^{5.7}$
Formaldehyde	0.5%	$\geq 10^{5.7}$
Glutaraldehyde (Cidex)	2.0%	$\geq 10^{5.7}$
Sodium hypochlorite	5.25%	$\geq 10^{4.7}$[c]
Chloroform	Undiluted	$\geq 10^{4.7}$[c]

[a] Virus was diluted 1:10 in the indicated chemical and immediately assayed for virus content.

[b] Original MVM titer = $10^{6.7}$ $TCID_{50}$/ml in secondary rat embryo cells.

[c] Chemical was toxic for cells at 1:10 dilution. Therefore, a 1:100 dilution was the initial dilution tested. At this dilution, there was no toxicity for the REC.

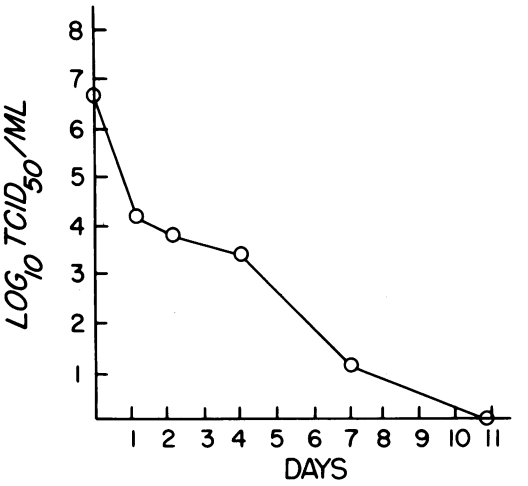

FIG. 1. *Acid inactivation of MVM. MVM was dialyzed against 0.15 N KCl-HCl solution at pH 2.0 at 4 C for 11 days. At intervals, samples were removed, the pH was adjusted to pH 7.2 by dialysis against Hanks balanced salt solution containing 0.02 M sodium phosphite, and the samples were titrated on secondary REC to determine the surviving* log_{10} *TCID*$_{50}$*/ml.*

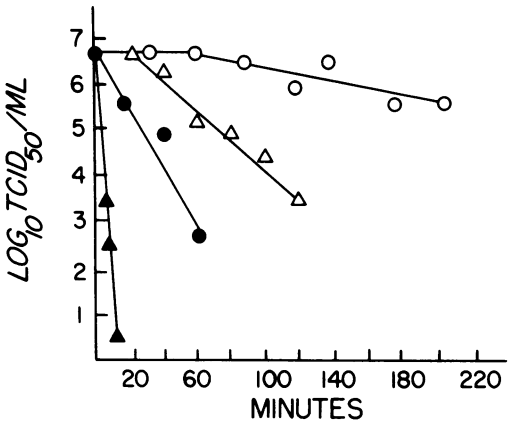

FIG. 2. *Heat inactivation of MVM. MVM was heated at the indicated temperatures, and samples were removed at intervals and titrated on secondary REC to determine the surviving* log_{10} *TCID*$_{50}$*/ml. Symbols:* (O) *35 C;* (Δ) *45 C;* (●) *60 C;* (▲) *100 C.*

virus titer was reduced to $10^{3.7}$ $TCID_{50}$/ml. Treatment at 35 C was less deleterious. No reduction in viral titer was observed after treatment for 60 min; then the titer gradually decreased to approximately $10^{5.7}$ $TCID_{50}$/ml by 220 min. Thus, treatment of MVM at 100, 60, or 45 C resulted in a loss of greater than 95% infectivity within 60 min, whereas treatment at 35 C required 180 min to effect a loss of 90% of the viral infectivity.

The virus was more stable at refrigerator and freezer temperatures. Storage for 10 weeks at 4 C resulted in a loss of viral titer to $10^{2.3}$ $TCID_{50}$/ml. After storage for 9 months in liquid nitrogen, there was no loss in MVM infectivity.

Inactivation of MVM by UV radiation. MVM was relatively sensitive to UV inactivation (Fig. 3). There was a linear decrease in the log_{10} of residual virus infectivity when the virus was irradiated with between 100 and 500 ergs/mm^2. The rate of UV inactivation was 1.2 log_{10}/100 ergs. However, whereas 100 ergs/mm^2 inactivated 98% of the infectivity, irradiation to 500 ergs/mm^2 was required to inactivate effectively the residual virus. UV inactivation, similar to the inactivation of MVM by acid conditions, or temperatures of 35, 45, or 60 C inactivated more than 90% of the virus within a short time, but invariably left a "persistent fraction" of the virus which was difficult to inactivate.

DISCUSSION

The susceptibility of MVM to inactivation by chemical disinfectants has not been reported in detail. Crawford (3) reported that 1.8% formaldehyde denatured the deoxyribonucleic acid of MVM. Parker et al. (9) reported that chloroform treatment had no effect on induction of MVM-specific fluorescence in infected cells; however, the concentration of chloroform used and the details of treatment were not reported. In the present studies, all of the chemical disinfectants used resulted in an immediate loss of MVM infectivity. The results with formaldehyde confirmed the data of Crawford (3) and demonstrated that as little as 0.5% formaldehyde completely inactivated measurable virus. Under our test conditions, chloroform also inactivated the virus. Data were not obtained on the susceptibility of MVM to phenolic derivatives or quarternary ammonium compounds, other commonly used disinfectants.

Few data are available in the literature concerning the stability of MVM to acid or alkaline conditions. Crawford (3) observed that the MVM hemagglutinin was not affected at pH 7.0 to 8.5, and Greene (4) demonstrated that the hemagglutinin of Toolan's H-1 virus (another Parvovirus) was stable at pH 2.0. However, it is not known whether the hemagglutinin of Parvoviruses is clearly associated with infectivity. In the present study, 99% of the infectivity of MVM was lost after exposure of the virus to pH 2.0 for 24 h, although a very small percentage of residual infectivity could still be detected after 7 days of this acid treatment.

In regard to thermal inactivation, many of the Parvoviruses appear to be relatively heat stable. Kilham and Oliver (7) demonstrated that Kilham's rat virus was still infective after heating at 80 C for 120 min. Hoggan et al. (5) demonstrated that the half-life of adeno-associated

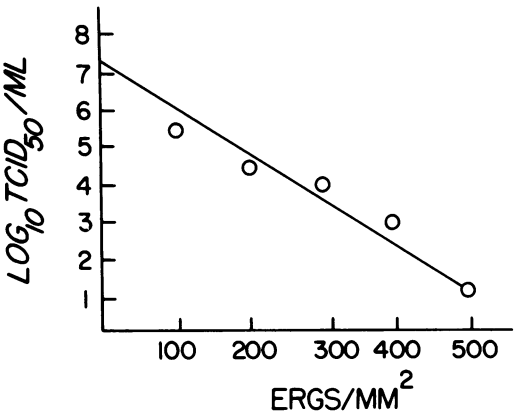

FIG. 3. *UV radiation inactivation of MVM. MVM was placed in 100- by 15-mm open petri dishes on a platform rocker. Varying doses of UV radiation were delivered to the samples, and residual virus was titrated on secondary REC to determine the* log_{10} *TCID*$_{50}$*/ml.*

virus infectivity was unaltered after heating at 56 C for 60 min; this virus appears to be very stable, since a definite loss of infectivity at 37 C occurred only after 180 days. MVM appears to be more heat sensitive than other Parvoviruses. In contrast to adeno-associated virus which had a half-life of 30 min at 60 C (5), MVM showed a half-life of only 15 min at 60 C. In addition, unlike the other Parvoviruses that have been studied, over 90% of MVM infectivity was lost within 3 h at temperatures of 35 C and above.

MVM also appears to be less stable than other Parvoviruses to storage at low temperatures. When stored at 4 C over a 10-week period, MVM lost 4.3 $\log_{10}$ of viral infectivity. In contrast, Poole (10) demonstrated that feline panleucopenia virus did not lose any infectivity when stored at 4 C for over 13 months. Toolan (16) has also reported that four of the H viruses had no loss of infectivity when stored at refrigerator temperature for over 6 years. MVM was stable when stored in liquid nitrogen for 9 months.

MVM was found to be more sinsitive to UV radiation than another Parvovirus, Kilham's rat virus. In the present study, 98% of virus infectivity was lost after irradiation for 100 ergs/mm^2, although an increased dose of 500 ergs/mm^2 was necessary to inactivate all detectable residual virus. From these data, the maximal radiation dose for 37% survival (D_{37}) of infectious MVM can be estimated to be 65 ergs/mm^2. This D_{37} is lower than that of 130 ergs/mm^2 reported for Kilham's rat virus, but is the same as the D_{37} reported for another single-stranded deoxyribonucleic acid virus, the bacteriophage X 174 (11).

Although the majority of MVM was inactivated by UV, heat, and acid treatment, a small "resistant population" was observed. These data may indicate the presence of a resistant mutant viral population, as has been reported for poliovirus (17). Bachman (1) failed to detect temperature-sensitive mutants with porcine Parvoviruses; however, the possibility of mutants cannot be eliminated in the present study. A resistant population may also be related to formation of aggregates of virus particles, or virus and cellular components, which limit complete destruction of virus (18). Crawford (3) indicated that MVM aggregates caused some difficulty in the initial characterization of the virus. The "resistant population" observed in the present studies occurred during inactivation of crude virus preparations consisting of cell culture fluids and disrupted cells. The results indicate that resistant fractions may occur if heat, UV radiation, or acid treatments are used to inactivate MVM in biological samples. The chemical disinfectants appeared to be more efficient in complete inactivation of MVM.

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